

RUNNING HEAD: Cochlear afferent pathway

Using Electrocochleography to Assess the Afferent Pathway in the Cochlea

Senior Thesis

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Table of Contents

Abstract	4
List of Figures	5
Introduction	6
Materials and Methods	8
Predicted Results	10
Results	12
Discussion	21
References	23

Abstract

Noise exposure has become a part of everyday life, but over exposure to loud daily noise may result in irreversible noise-induced hearing loss (NIHL). NIHL is a result of damage to the outer hair cells (OHCs) and spiral ganglion neurons (SGNs) of the cochlea- the key inner ear structures responsible for the sense of hearing. Currently, there are several diagnostic tests that are capable of detecting damage to the OHCs; however, no test exists that can detect damage or loss of SGNs. It would be desirable to have a diagnostic test to detect such damage due to the distortion effect that SGN loss can have on sound stimuli such as speech. The purpose of this study is to begin developing a diagnostic test that could be used to detect the damage or loss of SGNs over the 30-60 years of the typical span of adulthood. For the test to be viable for clinical use, it needs to be stable and consistent from one test session to the next. For the current project, every 4 weeks for a 24-week duration, the electrocochleography (EcochG) and Auditory Brainstem Response (ABR) of 7 rat subjects were measured and different components (the cochlear microphonic (CM and ABR Wave V) were analyzed to assess the health of the SGNs. Our results showed that the CM-ABR Wave V ratios were statistically unchanged at each of the monthly test times. Such a finding would mean that there is the possibility that the testing could be used to assess damage or loss of SGNs in the noise-exposed human population without concern that changes detected in the test are due to random fluctuations that occur between testing sessions.

List of Figures

Figure 1. Example response waveform elicited from a 5 kHz stimulus at 90 dB SPL.	11
Figure 2. ABR Wave V amplitude input-output functions for the 5 kHz stimulus.	13
Figure 3. ABR Wave V amplitude input-output functions for the 10 kHz stimulus.	14
Figure 4. ABR Wave V amplitude input-output functions for the 15 kHz stimulus.	15
Figure 5. ABR Wave V amplitude input-output functions for the 20 kHz stimulus.	16
Figure 6. ABR Wave V amplitude input-output functions for the 30 kHz stimulus.	17
Figure 7. CM-ABR Wave V amplitude ratio input-output functions for the 5 kHz stimulus.	18
Figure 8. CM-ABR Wave V amplitude ratio input-output functions for the 10 kHz stimulus.	19
Figure 9. CM-ABR Wave V amplitude ratio input-output functions for the 15 kHz stimulus.	20

Introduction

Noise has become part of everyday life in industrial America, and many people disregard or ignore the daily noises to which they are exposed. However, as harmless as these sounds may seem, it is important to understand the irreversible damage that can be caused by over exposure to intense noise. Every day, around 30 million Americans are exposed to noise levels considered to be at a dangerous level, while 10 million Americans suffer from irreversible noise-induced hearing loss (NIHL) (ASHA, 2013). These dangerous exposures can come from the most obvious causes of occupational noise: factory work, construction work, military use of firearms and explosives, etc. But dangerous exposures can occur from other, more subtle, sources: portable music players, traffic noise, power tools, etc. Without proper hearing protection, this over-exposure to noise at dangerous levels can result in permanent hearing loss due to destruction of the inner ear structures.

The inner ear structure responsible for the sense of hearing is the cochlea. The cochlea is a complex structure with numerous unique cell populations that combine to transduce sound signals into the electro-chemical communication of the nervous system. Within the cochlea, the two structures most heavily damaged by noise exposure are the outer hair cells (OHCs) (Henderson et al., 2006) and the spiral ganglion neurons (SGNs) (Kujawa and Liberman, 2006; 2009; Lin et al., 2011). There are several tests that can detect noise-induced OHC loss, including otoacoustic emissions and the puretone audiogram; however, no current testing is available to detect damage or loss of SGNs. A reliable test to determine SGN damage would be of great use for diagnosing the nature of an individual patient's NIHL. Loss of OHCs leads to a predictable and consistent series of hearing deficits, most notably a threshold shift to pure tonal sounds that can be easily assessed with a standard clinical audiogram. With damage to the SGNs, the impact

on hearing sensitivity is much more subtle. There can be massive damage to the SGNs without a significant change in pure tone thresholds on the audiogram, as evidenced by children with auditory neuropathy spectrum disorder (Starr et al., 1996). Processing of complex sounds, such as speech, and the ability to hear in background noise are slowly diminished in patients with SGN loss. Speech discrimination testing is an effective means of identifying patients in whom the SGNs are damaged, but the diagnostic challenge lies in the fact that there needs to be a considerable amount of damage to the SGNs before there are noticeable changes in speech discrimination ability. Therefore, by the time a diagnosis of probable SGN impairment can be made, the damage is often too severe for any effective treatment option to be considered. Further complicating the issue is that SGN damage has been shown to occur in animal models after noise exposures that had previously been believed to be non-hazardous (Kujawa and Liberman, 2006; 2009). Therefore, there may be a population of human patients who are experiencing SGN damage due to noise they believe to be non-hazardous, and there is currently no way to detect SGN damage before there is massive cell loss and significant resultant hearing impairment. Development of testing to identify SGN damage could further add to the understanding and solution of these individuals' problems.

The current study assessed the long-term stability of the ratio of the cochlear microphonic (CM) to Wave V of the auditory brainstem response (ABR). The CM is an electrophysiologic response derived from the responses of the outer hair cells, while Wave V of the ABR is a central auditory response generated by the lateral lemniscus and/or inferior colliculus of the central auditory nervous system. The underlying logic for creating a ratio of those two potentials is that the CM is immune to changes in the SGNs, while Wave V of the ABR will be reduced by

loss of input from the SGNs. Therefore, in cases of SGN loss, the CM-Wave V ratio should increase.

Materials and Methods

Auditory Evoked Potentials (AEPs) are measurements of nerve and/or muscle activity after the presentation of an auditory stimulus (Jewett and Williston, 1971). AEPs can be subdivided into tests based on latency of responses; these subdivisions are: Electrocochleography (EcochG), Auditory Brain Stem Response (ABR), Auditory Middle Latency Response (MLR), and Auditory Late Responses (ALR) (Martin & Clark, 2012). The current project focused on one component of EcochG; the CM. The other response analyzed was the ABR Wave V. The CM is a measurement of the receptor potentials of the OHCs (Hall, 2007) and is used to measure the displacement action of the basilar membrane (Yost, 2008). The ABR Wave V is the largest of the brainstem auditory evoked potentials and reflects activation of the lateral lemniscus and inferior colliculus (Hall, 2007). By combining these tests in a novel way, the goal was to be able to more specifically identify the locations of pathologies involved in hearing loss in relation to the spiral ganglion. These tests will help us assess the afferent pathways – pathways that carry impulses from periphery towards the nervous system (Martin & Clark, 2012), from the cochlea to the brain.

Importantly, the CM and ABR Wave V components have a directly proportional relationship between amplitude and intensity; this means that as the stimulus intensity increases, the amplitudes of the CM or ABR Wave V also increase. In conditions in which the SGNs are lost or damaged, the ABR Wave V amplitudes will be depressed due to a lack of cells contributing to the response. The CM is immune to changes in the SGNs, since it is a reflection of the OHC receptor potentials. Therefore, in conditions of SGN degeneration, the CM will

remain stable and the ABR Wave V will decline. The CM- ABR Wave V ratio will increase. Including the CM in conjunction with the ABR Wave V will allow more precise measurements of SGN decay to be made, without concern about test/re-test reliability.

In this experiment, 7 Fischer 344/NHsd normal hearing rats were tested. The animals' CM and ABR Wave V responses were recorded every 4 weeks. Although the term "ABR Wave V" is used here, that is a term derived from the human ABR. In the human ABR the largest wave, the one generated by the lateral lemniscus and inferior colliculus, is the fifth wave in the ABR complex. In the rat, it is the second wave in the complex, with the first wave being the compound action potential (CAP, See Figure 1). The term "ABR Wave V" is used in this experiment to connect it to the human ABR. The goal of the current study is to establish norms for this combination of tests and assess the test's reliability from month to month. This progression of testing will hopefully add to the knowledge of the relationship between the CM and ABR Wave V, along with supporting our search for testing that can specify particular cell pathologies (particular to the SGNs) that are related to hearing loss.

The 7 animals were anesthetized using inhalant isoflurane (4% for induction, 1.5% for maintenance, 1L/min O_2 flow rate). Sub-dermal platinum needle electrodes were used to record responses and were placed behind the contralateral pinna (non-inverting), behind the ipsilateral pinna (inverting) and behind the shoulder blade (ground). Toneburst stimuli were used at frequencies of 5, 10, 15, 20, and 30 kHz and responses were recorded. The stimuli were delivered from a speaker positioned 3 inches from the test ear of each animal. These acoustic stimuli were calibrated before each testing and BioSig RZ programming was used for data collection. Each toneburst had a duration of 1 ms with a 0.5 ms rise/fall time with no plateau. The signal level was decreased in 5dB steps beginning at 100 dB SPL and decreasing to 0 dB

SPL. The amplitudes of the CM and ABR Wave V were measured by cursoring the highest peak of each wave and the lowest negative trough after that peak (see Figure 1). A two-factor ANOVA (time x stimulus level) was used to analyze the ABR thresholds across the 7 animals and across the 6 different test stimuli.

Predicted Results:

With the current experiment, the goal was to establish whether the ratio of the CM and ABR Wave V, across multiple stimulus intensity levels and frequencies, is consistent over time. Hypothetically, if there were differences in the recording parameters from test to test (due to electrode placement or impedance, or depth of animals' anesthetic states) those differences should have affected the CM and ABR Wave V equally, and the CM-ABR Wave V ratio should have remained stable. Therefore, our predicted result was that the CM-ABR Wave V ratios would be statistically indistinguishable from each other at each of the monthly test times. If there was significant instability in the CM-ABR Wave V amplitude ratios over multiple tests, there is no future for this novel design in diagnostic testing for those with hearing loss.

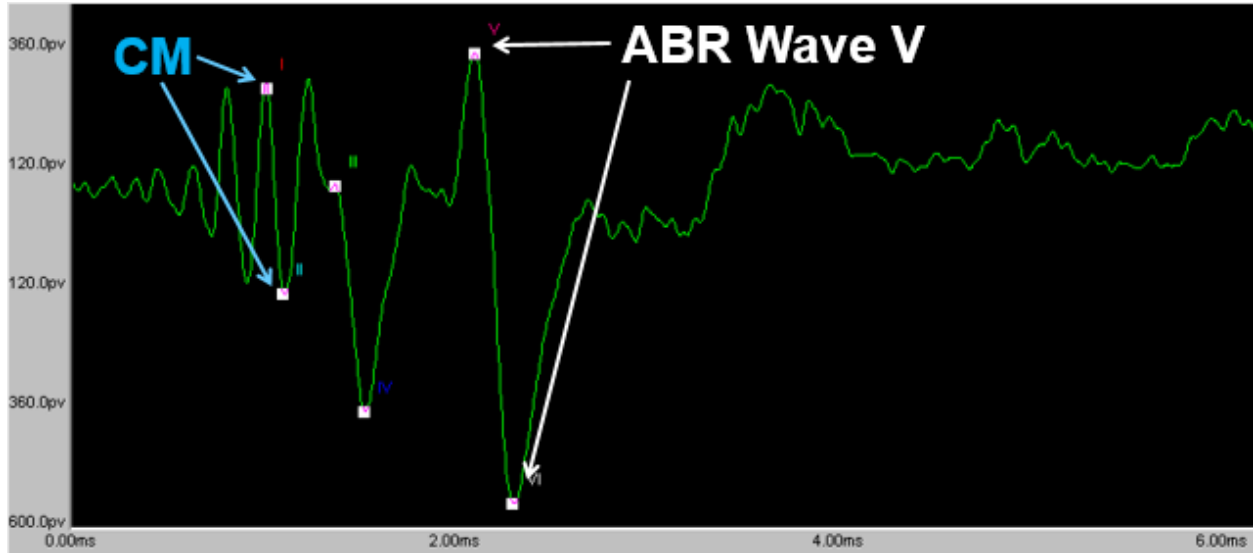


Figure 1: Example response waveform from a Fischer 344/NHsd normal hearing rat elicited from a 5 kHz stimulus at 90 dB SPL. ABR Wave V amplitude in this example was calculated by subtracting the amplitude of cursor VI from the amplitude of cursor V to calculate peak-to-peak amplitude.

Results

ABR Wave V amplitudes at each of the frequencies are displayed in Figures 2 through 6 showing the amplitudes of ABR Wave V responses over the 24 week period to the stimuli from 30 dB SPL to 100 dB SPL. Levels below 30 dB SPL were excluded from the analyses because few animals had consistent responses at those low levels. At each frequency (5, 10, 15, 20, and 30 kHz), there were no statistically significant differences in the ABR Wave V amplitudes from week to week.

Next, the CM-ABR Wave V ratios were assessed from week to week. Notice, only 3 frequencies (5, 10, 15 kHz) were assessed for this ratio. That is because the CM response disappeared or was too small to record at the 20 and 30 kHz levels. In Figures 7 through 9 the CM-ABR Wave V ratios are graphed displaying the results from week 0 to week 24. For this ratio response, there were no significant differences from month to month. There was a significant week x level interaction detected for the 5 kHz stimulus in which the Week 8 recording was significantly lower than the other weeks at 80 and 85 dB SPL

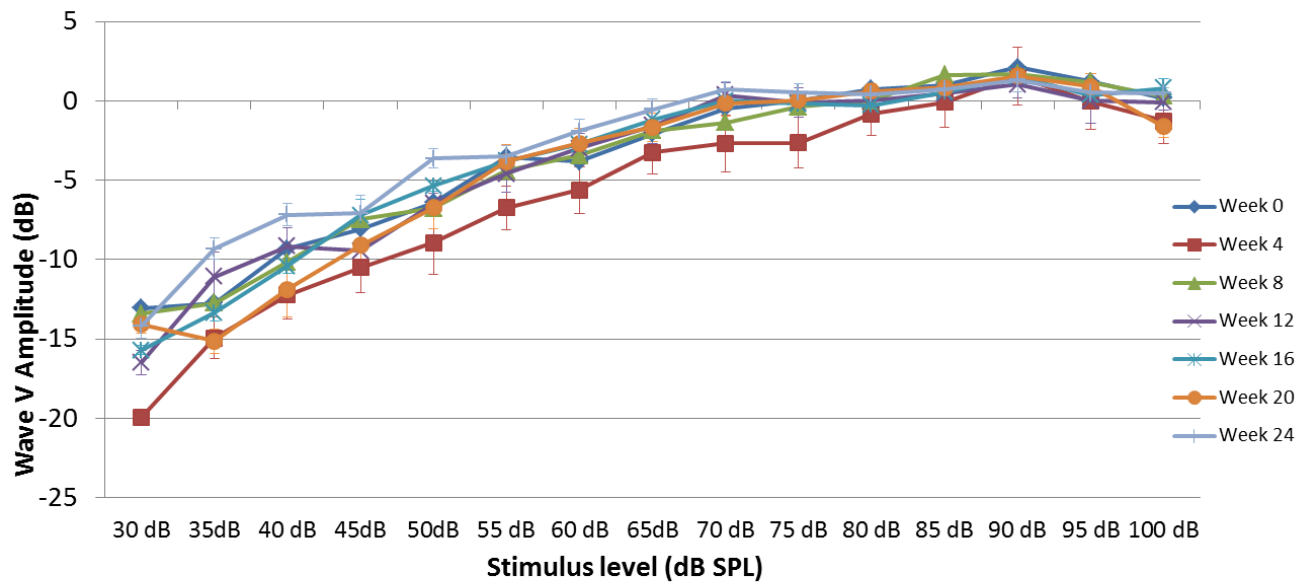


Figure 2: ABR Wave V amplitude input-output functions for the 5 kHz stimulus. No statistically significant differences between test weeks were detected.

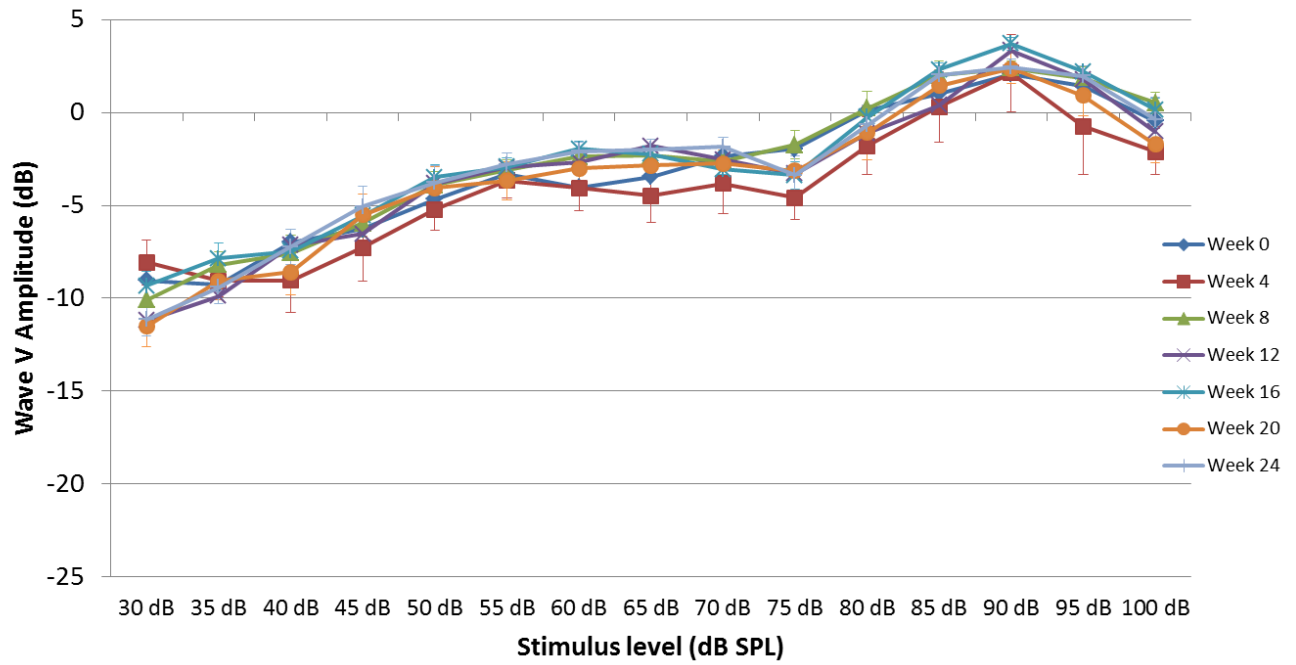


Figure 3: ABR Wave V amplitude input-output functions for the 10 kHz stimulus. No statistically significant differences between test weeks were detected.

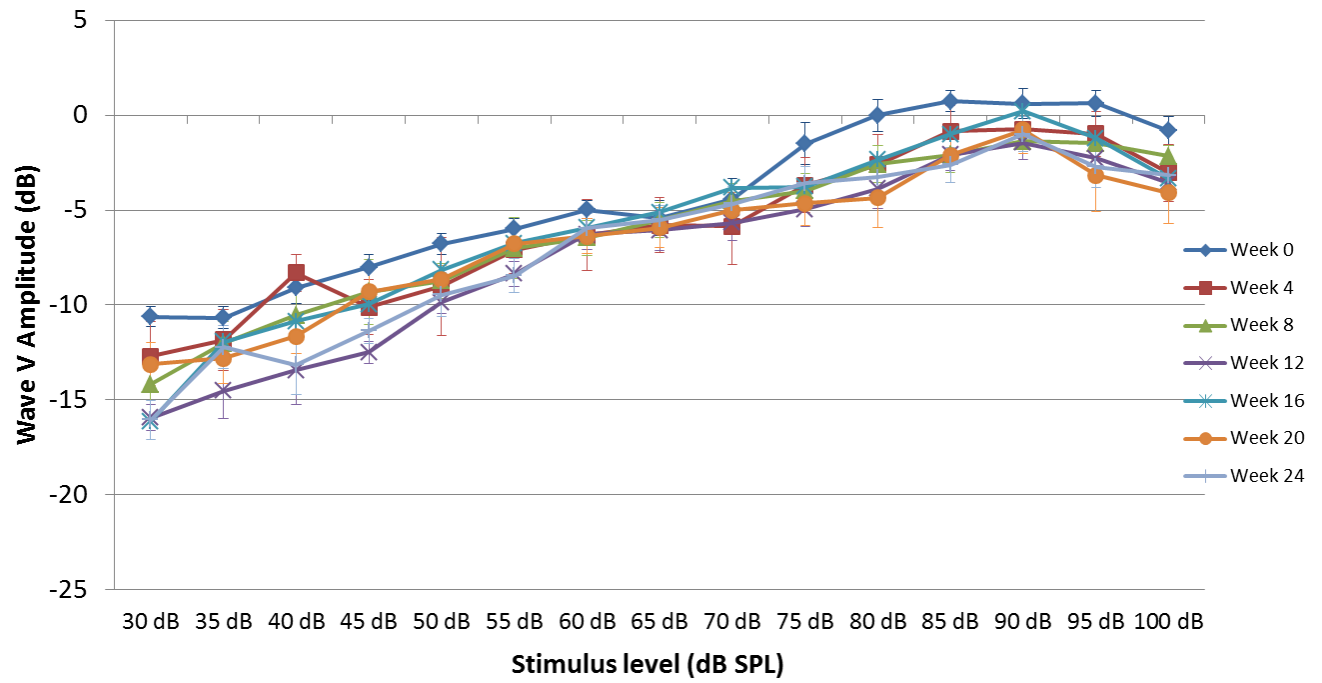


Figure 4: ABR Wave V amplitude input-output functions for the 15 kHz stimulus. No statistically significant differences between test weeks were detected.

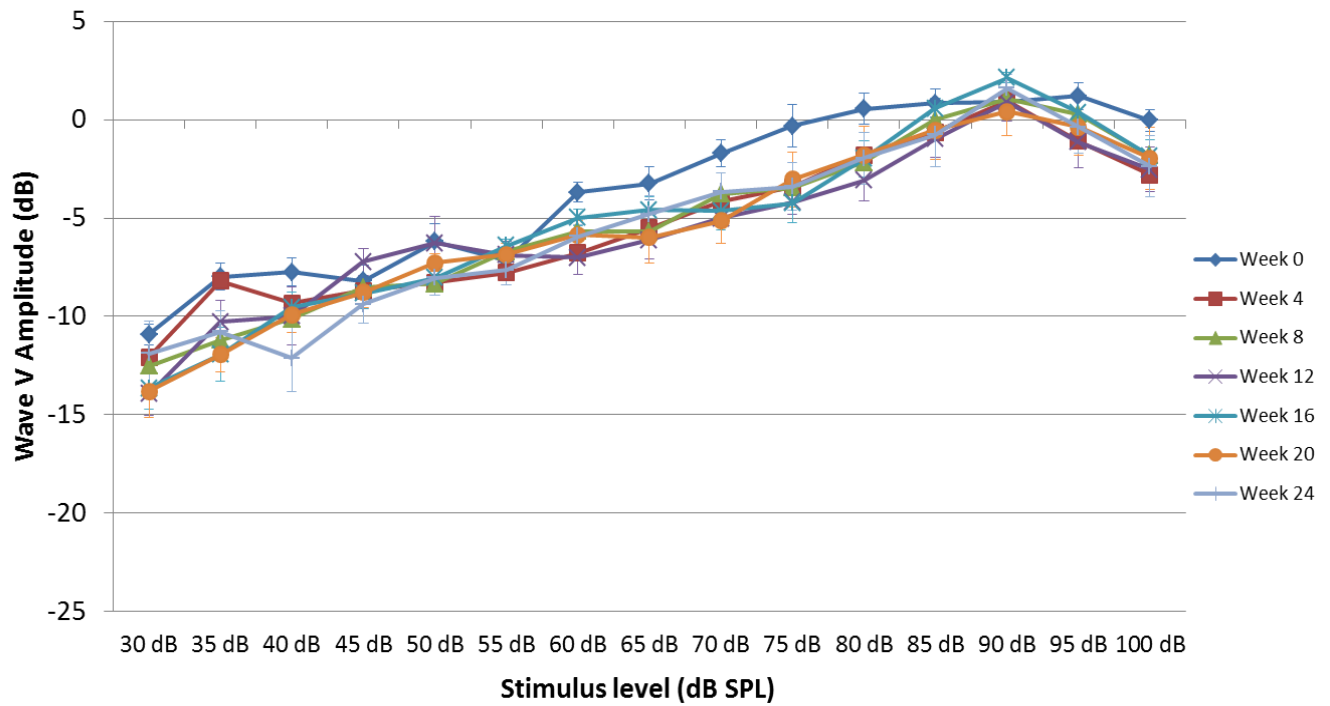


Figure 5: ABR Wave V amplitude input-output functions for the 20 kHz stimulus. No statistically significant differences between test weeks were detected.

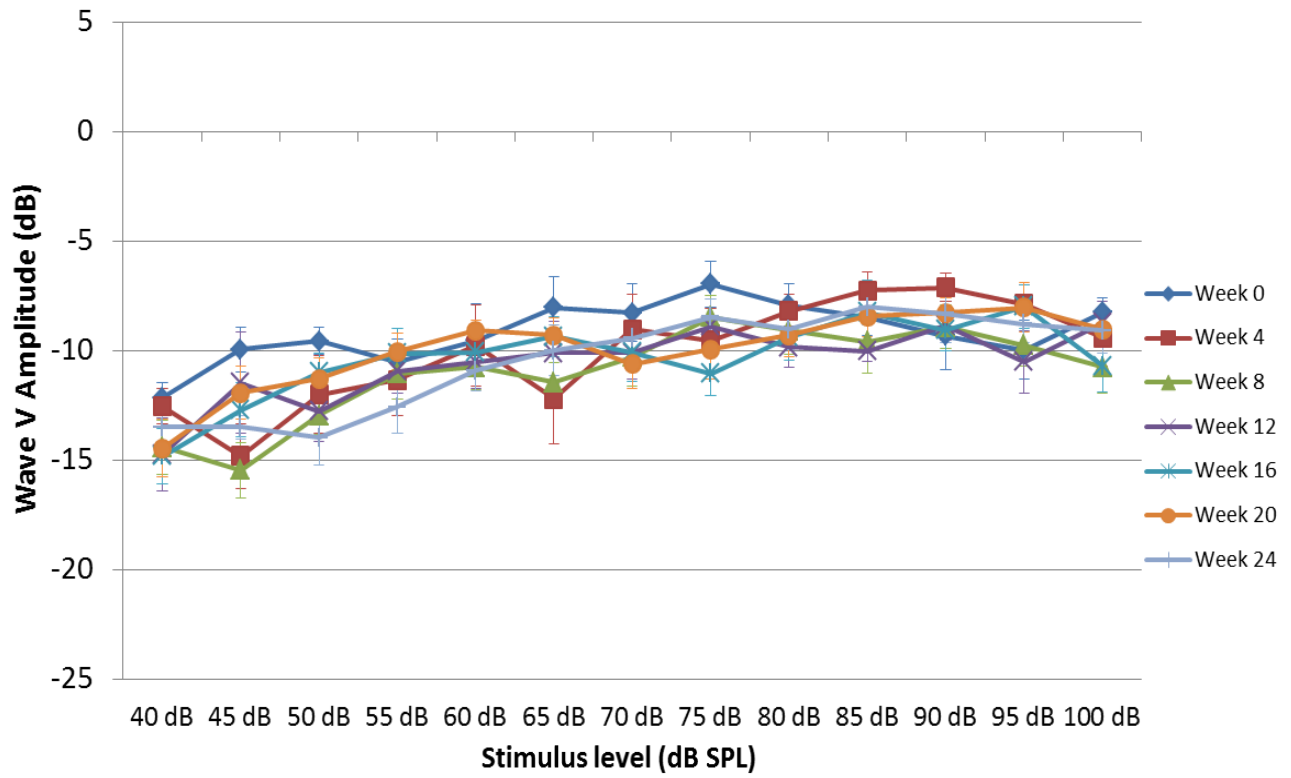


Figure 6: ABR Wave V amplitude input-output functions for the 30 kHz stimulus. No statistically significant differences between test weeks were detected.

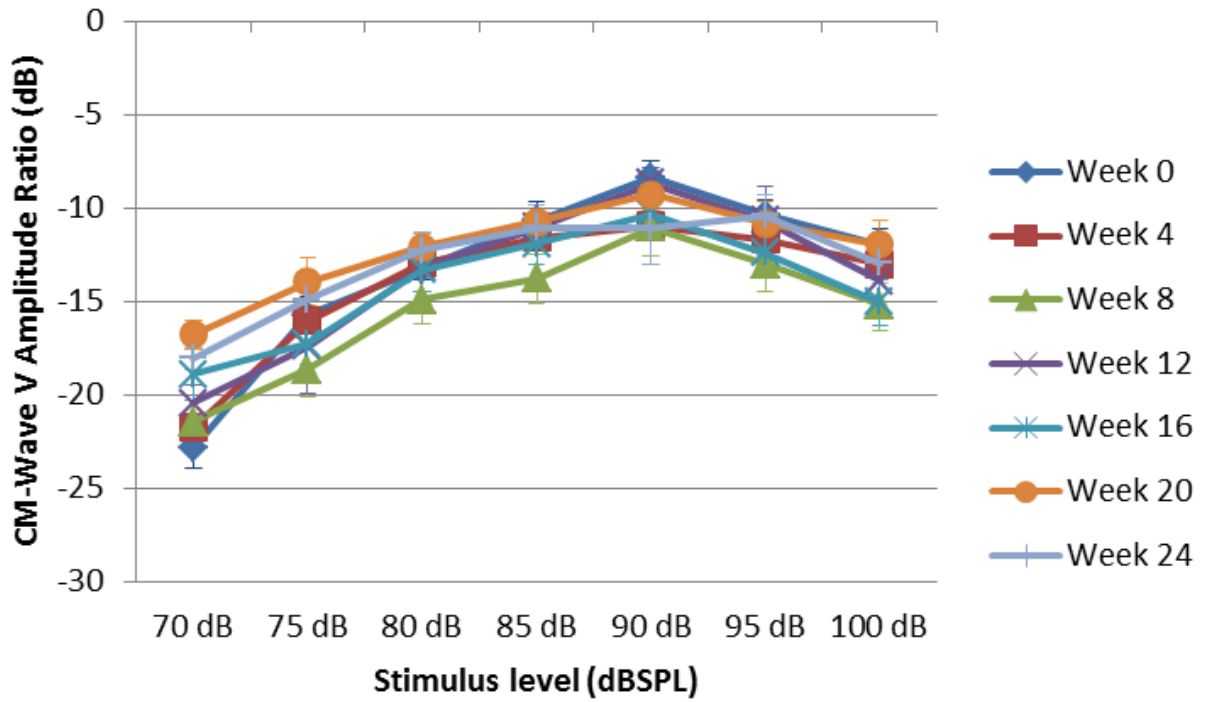


Figure 7: CM-ABR Wave V amplitude ratio input-output functions for the 5 kHz stimulus. A significant week x level interaction was detected, and Week 8 was lower than the other tests at 80 and 85 dB SPL.

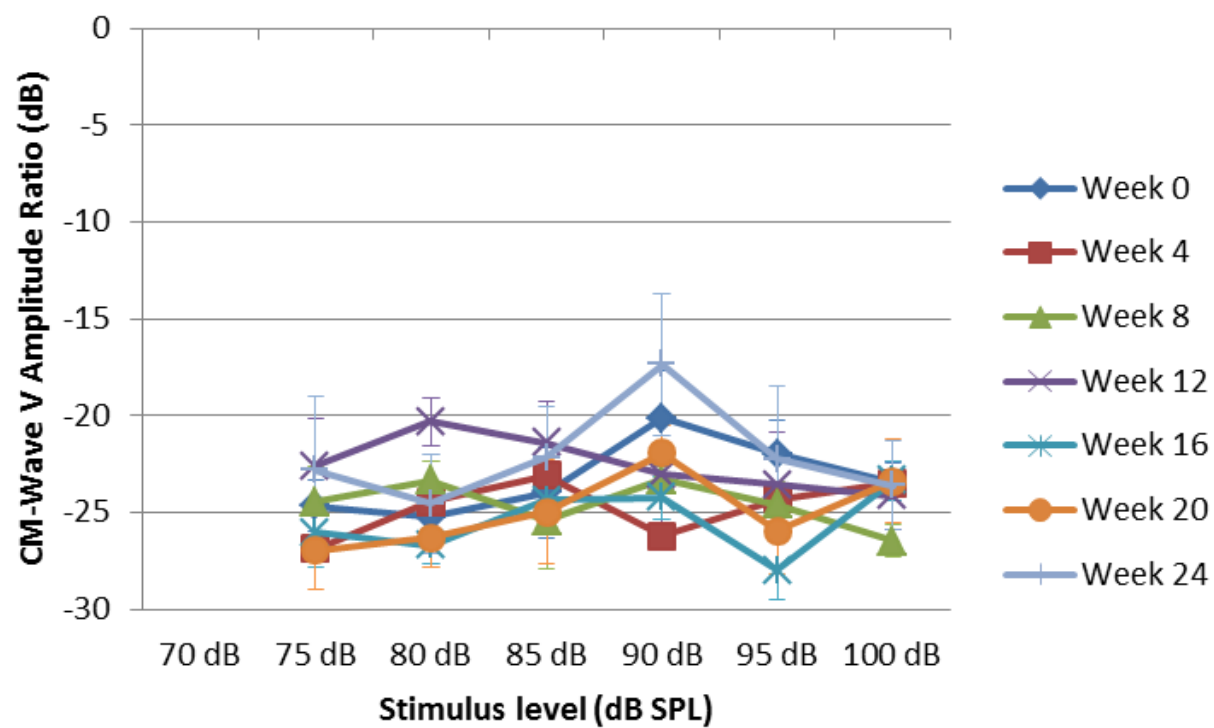


Figure 8: CM-ABR Wave V amplitude ratio input-output functions for the 10 kHz stimulus. No statistically significant differences between test weeks were detected.

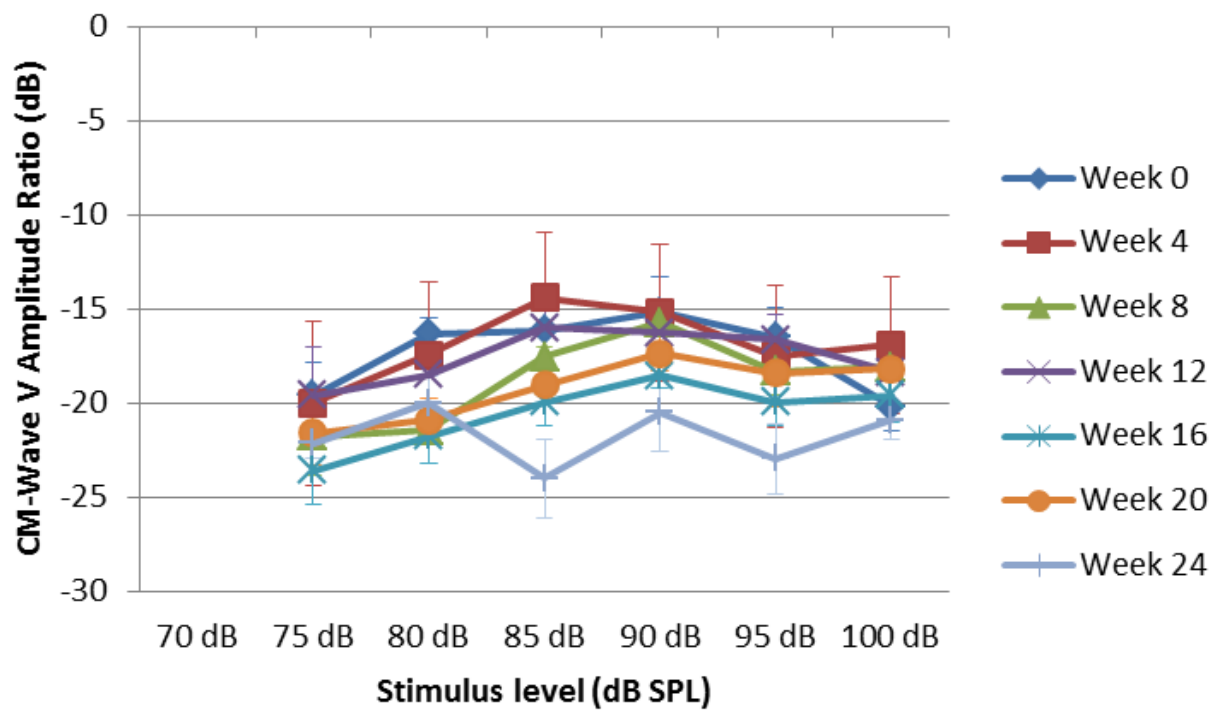


Figure 9: CM-ABR Wave V amplitude ratio input-output functions for the 15 kHz stimulus. No statistically significant differences between test weeks were detected.

Discussion

The results of this study indicate that the predicted result that the tests would be statistically indistinguishable from month to month was indeed correct. This conclusion can be drawn because the animals' Wave V amplitudes and CM-Wave V ratios did not change with any significance over the testing times. There was a change in the 5 kHz CM-Wave V ratio result at Week 8 at two stimulus intensity levels, but these changes did not persist over any of the test points thereafter. This indicates that those changes were simply an anomaly of that particular test point. The general stability of these measures over time indicates that there is a good test-retest reliability with these particular assessments. Good reliability is crucial for potential clinical use for testing in individual patients with cochlear damage, and this study indicated that these assessments may be useful clinically if they do prove to be sensitive to SGN damage.

The presence of the statistically-significant changes at the Week 8 time point does indicate that the test is susceptible to some random fluctuations. This would indicate that if the test is to be used in individual patients who have been exposed to noise, the test should be repeated at regular intervals before a firm diagnosis can be made. The test's ideal use is as a monitoring test for patients who experience noise exposure in the workplace or recreationally. The stability of the test seen in the current study is an indication that with repeated tests over time, those without SGN damage will be correctly identified as normal if a pre-exposure measurement has been taken, or comprehensive normative data can be acquired.

What is unknown from the current test is how the CM-Wave V ratio responds to noise exposures. That question is being addressed in other experiments. We hypothesize that in such experiments the CM-ABR Wave V ratios will increase in a noise-exposed population. This increase in the CM-ABR Wave V ratio will be the result of cochlear de-afferentation. The

current study might have benefitted from more animals being tested instead of the seven used in the study. In general, the variances were relatively low, indicating that more animals and greater statistical power would not have revealed any differences between test times.

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